

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A01H 5/10, C08B 30/00 A23L 1/308, 1/0522	A1	(11) International Publication Number: WO 94/03049 (43) International Publication Date: 17 February 1994 (17.02.94)
(21) International Application Number: PCT/AU93/00389 (22) International Filing Date: 30 July 1993 (30.07.93) (30) Priority data: PL 3894 31 July 1992 (31.07.92) AU PL 7266 12 February 1993 (12.02.93) AU (71) Applicant (for all designated States except US): GOODMAN FIELDER INGREDIENTS LIMITED [AU/AU]; Level 4, 230 Victoria Road, Gladesville, NSW 2111 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): McNAUGHT, Kenneth, J. [AU/AU]; 18 Marcella Street, North Epping, NSW 2121 (AU). MALONEY, Eric [AU/AU]; 169 Brisbane Street, Tamworth, NSW 2340 (AU). BROWN, Ian, L. [AU/AU]; 2 Melissa Avenue, Tamworth, NSW 2340 (AU). KNIGHT, Adrian, Timothy [AU/AU]; 18 Nundah Street, Lane Cove, NSW 2066 (AU).		(74) Agent: F.B. RICE & CO.; 28A Montague Street, Balmain, NSW 2041 (AU). (81) Designated States: AU, CA, JP, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS (57) Abstract Hybrid maize seeds which yield a starch having an amylose content of 80 % or more are disclosed as are starches having that amylose content. Compositions including these high amylose starches are also disclosed. Fractions of high amylose starches which have been formed on the basis of granule size are shown to have enhanced dietary fibre and/or resistant starch content. Such fractions enable the preparation of food compositions of enhanced dietary fibre and/or resistant starch content.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

- 1 -

HIGH AMYLOSE STARCH AND
RESISTANT STARCH FRACTIONS

Technical Field

This invention relates to high amylose content
5 starch, in particular to a maize starch having an amylose
content of more than 80% w/w. The invention further
relates to single, double and multiple cross maize
hybrids, particularly to a maize single cross F1 hybrid,
capable of producing grain having such a high amylose
10 content and to this grain.

The invention still further relates to fractions of
high amylose starch that are enriched in dietary fibre and
resistant starch content whilst claiming a high amylose
content.

15 Background Art

Most common starches contain approximately 25%
amylose and 75% amylopectin. Amylose is a linear glucose
polymer fraction, whilst amylopectin is a branched glucose
polymer fraction.

20 In the prior art, it has been recognized that
currently available commercial starch having an elevated
amylose content would impart certain desirable properties
to various compositions including films, foods and
industrial products. Accordingly, attempts have been made
25 in the prior art to produce high amylose content maize.
This is exemplified in AU-A-45616/89 wherein a maize seed
deposited as ATCC No. 40499 is disclosed as capable of
yielding a starch having an amylose content of up to 72%.

Typically, however, a commercial starch having an
30 amylose content of 55-65% would be regarded in the art as
having a high amylose content.

The present inventors whilst recognizing the utility
of the commercially available so-called high amylose
starches, have sought to produce a maize having a still
35 higher amylose content.

SUBSTITUTE SHEET

- 2 -

Disclosure of Invention

In the course of a breeding program, a single cross F1 hybrid maize seed was produced, which carried the ae amylose extender gene. This seed was found to be capable
5 of producing grain, in which the amylose content of the starch derived therefrom was in excess of 80%.

Accordingly, in a first aspect, this invention consists in a hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

10 In a second aspect, this invention further consists in a maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and deconstructurized and non-deconstructurized forms thereof.

15 In a third aspect, this invention still further consists in compositions including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and deconstructurized and
20 non-deconstructurized forms thereof.

In a fourth aspect, this invention still further consists in a process for the formation of a composition comprising including a maize starch selected from the group consisting of maize starch having an amylose content
25 of more than 80%, physically or chemically modified derivatives thereof and deconstructurized and non-deconstructurized forms thereof, in said composition.

In a fifth aspect, the present invention still further consists in a hybrid maize seed resulting from a
30 cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than
35 80%.

- 3 -

Starch granules from any botanical source are a heterogeneous mixture varying in physiological age and this affects their physical size, structure and properties. If the starch granules are physically separated according to their granule size, it has been noted by a number of authors that the properties of each size fraction are somewhat different. For example, Cluskey et al in Starke, 32, 105-109(1980) reported on the fractionation of dent corn and amylomaize starch granules. They found that an inverse relationship existed between granule size and iodine binding capacity in the amylomaizes. Thus, the percent apparent amylose found in the fractions of amylose V starch amounted to 40% for the largest size particles and 52% for the smallest particles.

The correlation between amylose content and size fraction has been observed by the present inventors in relation to high amylose starches of the type mentioned above and in co-pending patent application PL6537.

In this latter mentioned patent application, PL6537, it was disclosed that high amylose starches have a high dietary fibre or resistant starch content. More specifically, it was found that there was a correlation between amylose content and dietary fibre/resistant starch such that increasing levels of amylose above 55% were associated with increasing levels of dietary fibre/resistant starch.

Patent application PL6537 further disclosed the useful nature of such starches in the preparation of food compositions having an enhanced dietary or resistant starch content.

Based on the observations of

- (1) an association of dietary fibre and resistant starch with increasing levels of amylose; and
- (2) increasing amylose content with decreasing starch granule size,

- 4 -

it was to be expected that decreasing starch granule size fractions of high amylose starch would be associated with enhanced levels of dietary fibre and resistant starch.

Surprisingly, this was found to be incorrect. In fact it was found that there is an optimum starch granule size fraction which is intermediate in size and not necessarily associated with the highest amylose content fraction.

Accordingly in a sixth aspect, the present invention still further consists in a starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

In a seventh aspect, the present invention still further consists in a food composition having an enhanced dietary fibre and/or resistant starch content, including a starch fraction of enhanced dietary fibre and/or resistant starch content derived from a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

For the purpose of the description of this invention, "high amylose" means an amylose content (dsb) of 50% or more, preferably 70% or more, most preferably 80% or more. Particularly preferred amylose contents are 85% or more and 90% or more.

For the purposes of the description of the invention, the method by which amylose was determined is set out below.

- 5 -

METHOD: Apparent Amylose (Blue Value)

SCOPE: High Amylose Maize Starch

APPARATUS:

Defatting

- 5 Soxhlet extraction apparatus
Steam bath
Whatman thimbles, 25 x 80mm
Drying Oven 105°C
Desiccator

10 Amylose Determination

- Stoppered 50ml test tubes
Vortex mixer
Boiling water bath
Spectrophotometer (605nm, slit width 0.2mm)

15 **REAGENTS:**

Defatting

Methanol (AR Grade)

Amylose Determination

- Dimethylsulfoxide (HPLC Grade)
20 Iodine/Potassium iodide solution
3.0g iodine and 30g potassium iodide made
up to 1000mls with 0.1N sodium hydroxide
Methanol (AR Grade)
Amylose (Sigma Cat. No A0512)
25 Dried for 2 hours at 105°C prior to use.

PROCEDURE:

Defatting

- (1) Weigh 5 grams of starch into the thimble.
(2) Place the thimble in the Soxhlet apparatus.
30 (3) Extract the sample with methanol (200mls) for
20 hours
(4) Recover the thimble and dry in an oven at
105°C for 12 hours.

Amylose Determination

- 6 -

- (1) Accurately weigh starch (100.0 to 105.0mg) into the test tube.
- (2) Add methanol (1ml) and vortex mix.
- (3) Add DMSO (15mls), invert the test tube, and vortex mix.
- (4) Place the test tubes in a vigorously boiling water bath for 60 minutes.
- (5) Invert and vortex mix each test tube at 15 minute intervals during this period.
- (6) Add distilled water (15mls), invert and vortex mix. Place the test tube in the boiling water bath for a further 30 minutes.
- (7) Quantitatively transfer the contents of the test tube to a 100ml volumetric flask (use a funnel in the flask). Make the solution to volume with distilled water.
- (8) Transfer an aliquot (3mls) of this solution to a 100ml volumetric flask and add 90mls of distilled water.
- (9) Add Iodine/Potassium Iodide solution (1ml) to the diluted solution and immediately shake and mix thoroughly. Make to volume with distilled water.
- (10) Measure the absorbance of this solution at 605 nm compared to a blank consisting of Iodine/Potassium Iodide solution (1ml) diluted to 100mls with distilled water in a volumetric flask.

CALCULATIONS:

For native starches:

$$\% \text{ Amylose dsb} = \frac{\text{Absorbance} \times 13}{\text{wt. sample dsb}}$$

* dsb = dry solids basis.

The method by which starch was separated from the maize grain was as follows:-

- 7 -

1. Prepare 200g meal by grinding through the 2mm then the 1mm screen of the Retsch Mill.
2. Wet thoroughly, stirring by hand, with 600ml 0.1N NaOH.
3. Add 2,200ml 0.1N NaOH and blend 5 minutes at 2/3 speed with the Ultra Turrax.
4. Sieve over 44u screen.
5. Return sieve overs with 1L water and blend for another 3 minutes, if necessary.
6. Sieve over 44u screen.
- 10 7. Centrifuge filtrate at 3000 rpm for 15 minutes. Decant. Wipe out the neck of the bottle with a tissue to remove fat.
8. Reslurry starch (centrifugate) with 200ml water, i.e. 50ml in each of 4 tubes. Centrifuge.
- 15 9. Remove starch from centrifuge tubes with about 250ml water.
10. Adjust pH of starch slurry to 6.0-6.5 with 0.5N HCl. Filter again over 44u screen, if necessary.
11. Buchner filter and air dry.

20 Modes for Carrying out the Invention

In order to better understand the nature of this invention, a number of examples will be described.

Brief Description of Drawings

Fig. 1 is a gel permeation chromatography molecular weight profile of a number of maize starches;

Fig. 2 is a viscograph of a number of maize starches in water;

Fig. 3 is a viscograph of a number of maize starches in base; and

30 Fig. 4 is a graph of total dietary fibre versus average starch granule size.

Maize Seed

A range of parental lines of maize seeds were obtained from High Yield Seed Co, Tamworth, Australia.

35 Non-limiting examples of these parenting lines included

- 8 -

G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144.

Hybrids were produced by crossing inbred lines
5 carrying the ae amylose extender genes. These inbred lines were selected for combining ability and identified as specific female and male parents to produce the hybrids. Conventional breeding methods and techniques were used in developing inbred lines with repetitive
10 amylose assays to ensure the transfer of recessively inherited ae gene.

One particular cross between male G116 and female G121 resulted in a F1 hybrid, referred to as Code 008 and deposited with the American Type Culture Collection
15 (ATCC), 12301 Parklawn Drive, Rockville, MD20853, U.S.A., under the designation 75182 on 15 January 1992. This hybrid yielded grain the starch of which was found to have an amylose content in excess of 80%.

Based on the disclosure of this invention, the person
20 skilled in the art would expect that hybrids resulting from further crosses of the parental lines mentioned above will yield starch having an amylose content in excess of 80%.

In fact experimental hybrids have yielded starches
25 obtained from crosses between the above mentioned parent lines having high amylose contents. Set out below is a summary of the relevant crosses with amylose content in % bracketed.

- 9 -

	<u>FEMALE</u>	<u>MALE</u>	<u>HYBRID</u>
	1. G117 (81.6)	G116 (82.2)	G117 x G116 (83.3)
	2. G116 (82.2)	G122 (89.6)	G116 x G122 (80.5)
	3. G118 (94.3)	G122 (89.6)	G118 x G122 (85.9)
5	4. G120 (94.6)	G122 (89.6)	G120 x G122 (80.4)
	5. G122 (89.6)	G120 (94.6)	G122 x G120 (81.9)
	6. G122 (89.6)	G140 (92.2)	G122 x G140 (85.4)
	7. G128 (71.5)	G129 (61.8)	G128 x G129 (82.8)
	8. G140 (93.2)	G121 (94.7)	G140 x G121 (93.0)
10	9. G140 (92.2)	G144 (60.4)	G140 x G144 (85.3)
	* 10. G139W (71.9)	G136W (93.4)	G139W x G136W (95.7)
	11. G121 (94.7)	G126 (82.2)	G121 x G116 (85.0)

* W = White seed.

15

Experiments conducted using Code 008 seed have shown that the climatic and agronomic conditions under which the maize is grown will have a significant effect on the amylose content. Specifically, it has been found that seed
 20 cultivated under irrigation near Tamworth, Australia (latitude 31.1°S) in an early crop and a late crop yielded starch having an amylose content respectively of 85.0% and 90.1%. Similarly, a crop cultivated at Finley, Australia (latitude 35.6°S) yielded starch having an amylose content
 25 of 94.8%. By contrast, the same seed when cultivated under irrigation at Giru, Australia (latitude 19.5°) yielded a starch having an amylose content of 78.6%.

Accordingly, a preferred embodiment of this invention comprises a maize seed deposited with the ATCC and
 30 designated 75182.

A further preferred embodiment of this invention comprises a maize starch having an amylose content of 85.0% or more, most preferably 90.1% or more.

To further characterize the maize starch derived from
 35 Code 008 grain, molecular weight profiling by gel

- 10 -

permeation chromatography has been performed. The method by which this was done is set out below whilst the results are shown in the accompanying Figure 1. For comparative purposes, two commercially available maize starches, HA

5 Class V and HA Class VII are shown.

METHOD: Gel Permeation Chromatography of Starch

SCOPE: Starch

APPARATUS:

Sample Preparation

10 Screw capped test tubes (50ml)
Boiling water bath
Microcentrifuge (Eppendorf 5415)
Desiccator

HPLC

15 Column Alltech GPC High MW Polar 5U
(Cat. No. 100586)
Detector Waters 410 Refractive Index
Detector (X 128 35°C)
Pump Waters 600 E
20 Injector Waters 712 WISP
Column Heater (Set at 25°C)
Software Maxima 825 (V 3.3)

REAGENTS:

25 Dimethyl sulfoxide (Chrom AR HPLC Grade -
Mallinckrodt)
Dimethyl formamide (Chrom AR HPLC Grade -
Mallinckrodt)
Pullulan Molecular Weight Standards - Showa
Denko (ex Edward Instruments)

30 HPLC Mobile Phase - DMSO:DMF (20:80)

SAMPLE PREPARATION:

Standards

(1) The pullulan molecular weight standards need to
be weighed into the screw capped test tubes in
35 the following manner:

- 11 -

Tube 1 - 5.0mg each of P800, P100, P10 and glucose

Tube 2 - 7.0mg each of P400, P50 and P5

Tube 3 - 7.0mg each of P200, P20 and maltotriose.

- 5 (2) Add DMSO (4mls) to each tube and tightly seal it.
- (3) heat the tubes in the boiling water bath for 5 minutes to dissolve the pullulan.
- (4) Remove and cool the test tube to room temperature.
- 10 (5) Add DMF (16mls) and mix well.
- (6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
- (7) Remove the top 1ml of solution from each centrifuge tube and place in a WISP vial.

15 **Samples**

- (1) Accurately weight the sample (50.0mg) into a screw capped test tube.
- (2) Add DMSO (10mls).
- (3) Heat in a boiling water bath for 60 minutes.
- 20 (4) Remove and cool the test tube to room temperature.
- (5) Add DMF (40mls) and mix well.
- (6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
- 25 (7) Remove the top 1ml of solution from each centrifuge tube and place in a WISP vial.

HPLC Preparation

- (1) Prior to fitting the column, pump water (100mls) through the HPLC.
- 30 (2) Prepare the mobile phase and pump 50mls through the HPLC. Ensure that the WISP is purged during this stage.
- (3) Adjust the flow rate of 0.2ml/minute and connect the column.
- 35 (4) Allow the column to equilibrate overnight.

- 12 -

- (5) Prior to the injection of samples, purge the WISP and then gradually increase the flow rate to 1.5mls/minute.
- (6) Set the column heater to 25°C.
- 5 (7) Inject the standards and samples - 100 μ l injection volume.
- (8) After samples have been analysed turn the column heater off and reduce the flow rate of 0.2mls/minute.
- 10 (9) Disconnect the column.
- (10) Wash the system with water overnight at 0.5mls/minute.
- (11) Wash the system with methanol (200mls).

Viscographs have also been prepared comparing maize
15 starch from Code 008 (designated Gelose 80) with Gelose 50 and Gelose 70. Figure 2 shows the viscosity profile under alkaline conditions whilst Figure 3 shows the viscosity profile in water.

Maize Starch

20 The maize starch of the first aspect of this invention having an amylose content of more than 80% may be used in a variety of compositions known in the art. The usefulness of the starch is believed to be a result of the higher content of more linear molecules. This seems
25 to impart physical properties which tend towards those of conventionally used synthetic plastics materials. Consequently, films formed from the starch of the invention have higher tensile strengths and are good oxygen barriers. The starch is also easier to process on
30 existing synthetic plastics materials equipment such as blow moulding and injection moulding machines.

Furthermore, this starch may be physically modified or chemically modified to produce a variety of derivatives well known in the art. These starches may also be used in
35 a variety of compositions.

- 13 -

Finally, this starch may also be used in processes and compositions requiring the starch to be destructured within the meaning of that term defined in EP0118240.

- Some non-limiting examples of compositions in which the maize starch of this invention in all of its forms, could be used include:
1. Corrugating adhesives.
 2. Sausage skins.
 3. Confectionery.
 4. Other food compositions where the enhanced gel strength of the starch would be advantageous.
 5. Films, either alone or laminated with polymers such as ethylenevinylalcohol to achieve both gas and water barrier properties.
 6. Biodegradable and controlled release matrices and methods for forming and using these matrices as disclosed in PCT/AU90/00422, the contents of which is incorporated herein by way of reference.
 7. Shaped articles, processes for forming shaped articles and methods for using shaped articles as disclosed in PCT/AU90/00237, the contents of which is incorporated herein by way of reference.
 8. Coextrusions with synthetic polymers.
 9. Intermediate products such as pellets and rods, formed for example by extrusion, and including combinations of starch with one or more natural or synthetic polymers, plasticizers, colourants and other additives.
 10. Other blends of starch with natural or synthetic polymers to obtain enhanced structural properties.

Starch Fractions

The starches of the sixth and seventh aspects of this invention may originate from a number of sources including cereals such as maize, barley, wheat and legumes, providing that the starch content of such sources is high in amylose.

- 14 -

To fractionate the starch granules, there are a number of methods known in the art including dry powder sieving, hydrocyclone classification, air classification and differential sedimentation. A person skilled in the art would be readily able to choose an appropriate method depending on the source material and other relevant factors.

Although the size fraction of enhanced dietary fibre and/or resistant starch may vary, the example that follows describes the work that was done by the present inventors in relation to a maize starch sample. Based on this disclosure, a person skilled in the art could readily repeat this work using other starch sources to identify an appropriate fraction.

Once the starch has been appropriately fractionated, the fractions having enhanced dietary fibre and/or resistant starch content may be processed to obtain starch having further increased dietary fibre and/or resistant starch content using entirely conventional methods well known in the art. An example of the fractionation will now be described.

Fractionation of Maize Starch by Granule Size

A high amylose maize starch - High Amylose 80(10/91) was fractionated into seven subsamples based on granule size using the aqueous differential sedimentation procedure described by Cluskey et al (1980). This method was chosen since it minimised damage to the starch, did not introduce any residues and it was indicated that exposure of the starch granules to distilled water for long periods of time did not affect their integrity. Each subsample was weighed, measured for average granule size and the apparent amylose content, total dietary fibre and resistant starch determined. Each starch sample (60 grams) was separated into the seven fractions which were freeze-dried and weighed on a Mettler PE 3600 top pan

- 15 -

balance. A scanning electron microscope was used to visually check the uniformity of the size distribution of the granules in each fraction.

Each fractionated starch sample was analysed for granule size according to the method described below. Apparent amylose content was determined using the method described above. Dietary fibre and resistant starch (McCleary et al) were determined using the methods disclosed in co-pending application PL6537.

Granule size was determined using a Malvern Master Sizer which utilises a He-Ne laser (632.8nm) with a maximum output of 5mW CW. In this method a starch slurry was made using approximately 15mL of distilled water in a 50mL beaker. The slurry was sonicated for 4 minutes. The slurry was then introduced into the stirred cell and the obscuration value adjusted using distilled water to 0.20. The slurry was allowed to stir for a further 2 minutes before readings were taken. Four readings were taken for each sample in order to check the stability of the readings being obtained.

Results

In Table 1 set out below, there is shown the results (the average of two separate fractionations, together with the range of analytical results) obtained for each of seven particle size fractions. These results are graphically presented in Fig.4, from which it is particularly evident that the level of resistant starch and dietary fibre is significantly increased between the second and fifth fractions, ie, 10.2-7.6 microns. Thus, if those starch fractions were to be segregated from the original starch sample, only 46.9% of the solids would need to be removed to produce a fraction in which the resistant starch was increased by 36% and dietary fibre by 24%.

Although the starch fractions of the invention are

TABLE 1Fractionation of High Amylose 80 (10/91) Maize Starch by Granule Size

	Amount in Fraction (%) dsb	Average Granule Size (microns)	Apparent Amylose Content (%) dsb	Total Dietary Fibre (%) dsb	Resistant Starch (%) dsb
High Amylose 80 - 10/91	100.00	10.0	85	33.4	18.1
Fraction 1	35.6 \pm 1.1	12.3 \pm 0.5	80 \pm 0	31.4 \pm 1.5	17.7
Fraction 2	15.0 \pm 2.6	10.2 \pm 0.1	83 \pm 1	38.3 \pm 2.0	16.4
Fraction 3	13.0 \pm 1.1	9.1 \pm 0.2	85.5 \pm 0.5	41.3 \pm 0.3	22.8
Fraction 4	14.9 \pm 1.0	8.3 \pm 0.1	85.5 \pm 0.5	39.4 \pm 4.1	24.6
Fraction 5	10.2 \pm 1.6	7.6 \pm 0.1	88.5 \pm 0.5	37.2 \pm 1.3	18.9
Fraction 6	7.0 \pm 1.6	7.2 \pm 0.1	89.5 \pm 0.5	31.3 \pm 2.4	21.7
Fraction 7	4.3 \pm 2.7	6.8 \pm 0.2	89	28.1	10.1

- 17 -

high in dietary fibre and/or resistant starch, it should also be appreciated that another important property is that these fractions are "naturally" derived. This arises out of the fact that the fractions are prepared using a physical means of separation. No chemical or other treatments are required in order to produce starch fractions having a high dietary fibre and/or resistant starch content. Such a property is of particular importance in food applications in that no regulatory approval would be required in order to incorporate such materials in food compositions.

The person skilled in the art will readily appreciate that the starch fractions of the invention having the enhanced dietary fibre and/or resistant starch content may be used in a variety of food compositions. Such uses are disclosed, for example, in co-pending application No PL6537.

Whilst it is not as yet known why the fractions of the invention have enhanced dietary fibre and/or resistant starch content, it will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described without departing from the spirit or scope of the invention as broadly described. Accordingly, the Example based on a sample of high amylose maize starch is to be considered in all respects as illustrative and not restrictive.

The person skilled in the art will readily appreciate that the maize starch of the invention both in its native form, and the other forms mentioned above will have many applications additional to those mentioned.

It will also be appreciated by those skilled in the art that numerous variations and modifications may be made to this invention without departing from the spirit or scope thereof as broadly described.

- 18 -

CLAIMS:

1. A hybrid maize seed capable of producing a starch having an amylose content of more than 80%.
2. A hybrid maize seed as in claim 1 obtained from a
5 cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than
10 80%.
3. A hybrid maize seed as in claim 2 selected from the group consisting of the following crosses: G117 x G116, G116 x G122, G118 x G122, G120 x G122, G112 x G120, G122 x G140, G128 x G129, G140 x G121, G140 x G144,
15 G139W x G136W and G121 x G116.
4. A hybrid maize seed as in any one of claims 1 to 3 wherein the seed yields a starch having an amylose content of 85.0% or more.
5. A hybrid maize seed as in claim 4 wherein the seed
20 yields a starch having an amylose content of 90.1% or more.
6. A hybrid maize seed as in claim 5 wherein the seed yields a starch having an amylose content of 94.8% or more.
7. A hybrid maize seed as in claim 3 deposited as ATCC 75182.
- 25 8. A maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.
9. A maize starch as in claim 8 having an amylose
30 content of 85.0% or more.
10. A maize starch as in claim 9 having an amylose content of 90.1% or more.
11. A maize starch as in claim 10 having an amylose content of 94.8% or more.
- 35 12. A composition including a maize starch selected from

- 19 -

the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and deconstructurized and non-deconstructurized forms thereof.

- 5 13. A composition as in claim 12 wherein the maize starch has an amylose content of 85.0% or more.
14. A composition as in claim 13 wherein the maize starch has an amylose content of 90.1% or more.
15. A composition as in claim 14 wherein the maize starch
10 has an amylose content of 94.8% or more.
16. A starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch, the amylose content of which is 50% or more, which has been fractionated according to granule size to yield a
15 fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.
17. A starch fraction as in claim 16 wherein the high amylose starch is selected from the group consisting of
20 maize, barley, wheat and legumes.
18. A starch fraction as in claim 16 or claim 17 wherein the amylose content of the high amylose starch is 70% or more, preferably 80% or more.
19. A starch fraction as in claim 18 wherein the amylose
25 content of the high amylose starch is 85% or more, preferably 90% or more.
20. A starch fraction as in any one of claims 16 to 19 wherein the fractionation is by dry powder sieving, hydrocyclone classification, air classification or
30 differential sedimentation.
21. A starch fraction as in any one of claims 16 to 20 wherein the dietary fibre content of the fraction is increased by about 24% or more and the resistant starch content of the fraction is increased by about 36% or more
35 over the high amylose starches.

- 20 -

22. A starch fraction as in any one of claims 16 to 21 wherein the average granule size of the fraction is from about 10.2 to 7.6 microns.

23. A food composition including a starch fraction as
5 claimed in any one of claims 16 to 22.

1/4

High Amylose Maize Starches

GPC Analysis

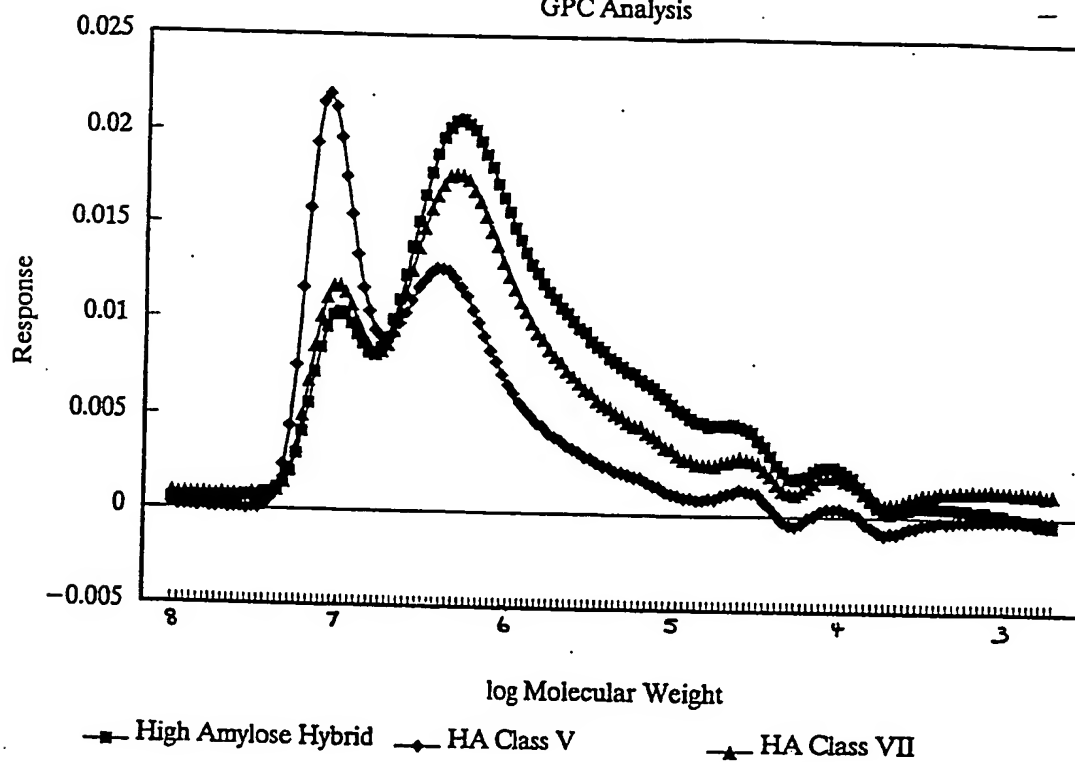


FIG. 1

SUBSTITUTE SHEET

GELOSE VISCOGRAPHS

IN WATER

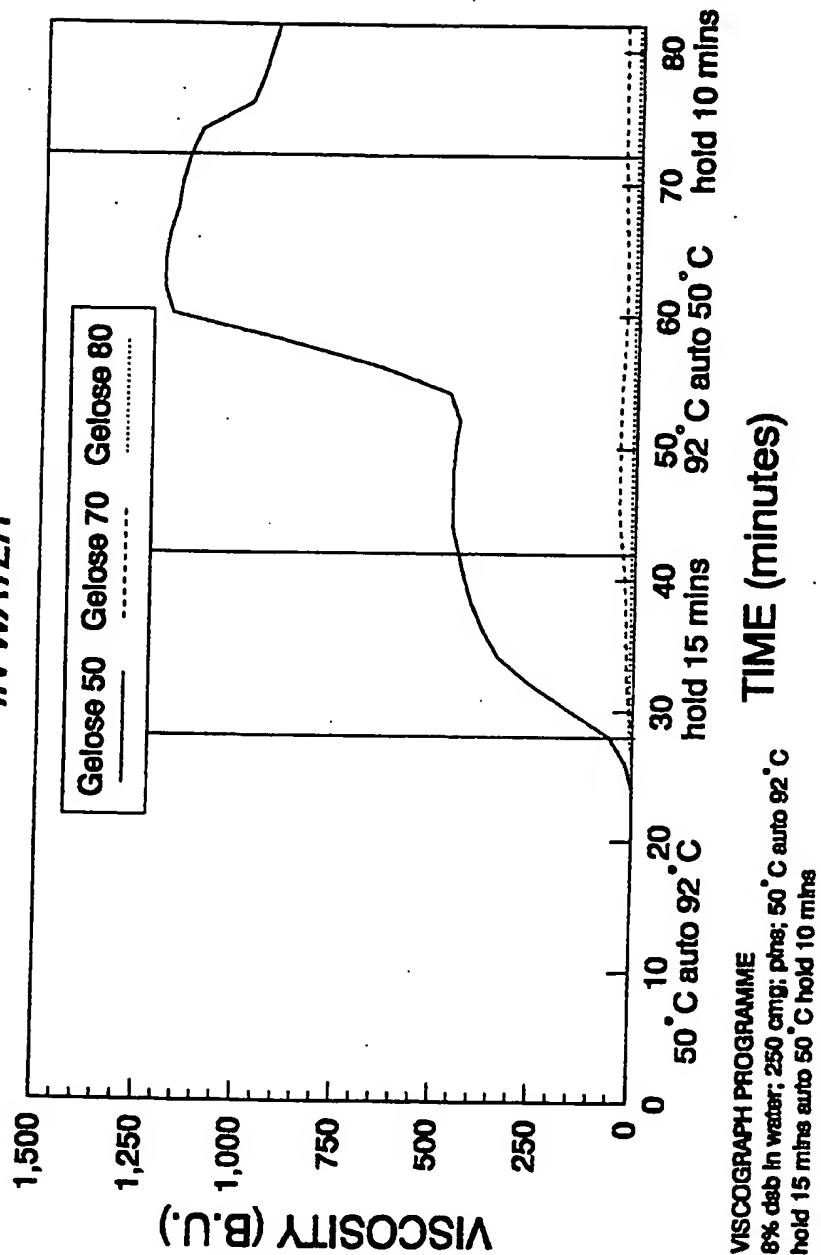


FIG. 2

3/4

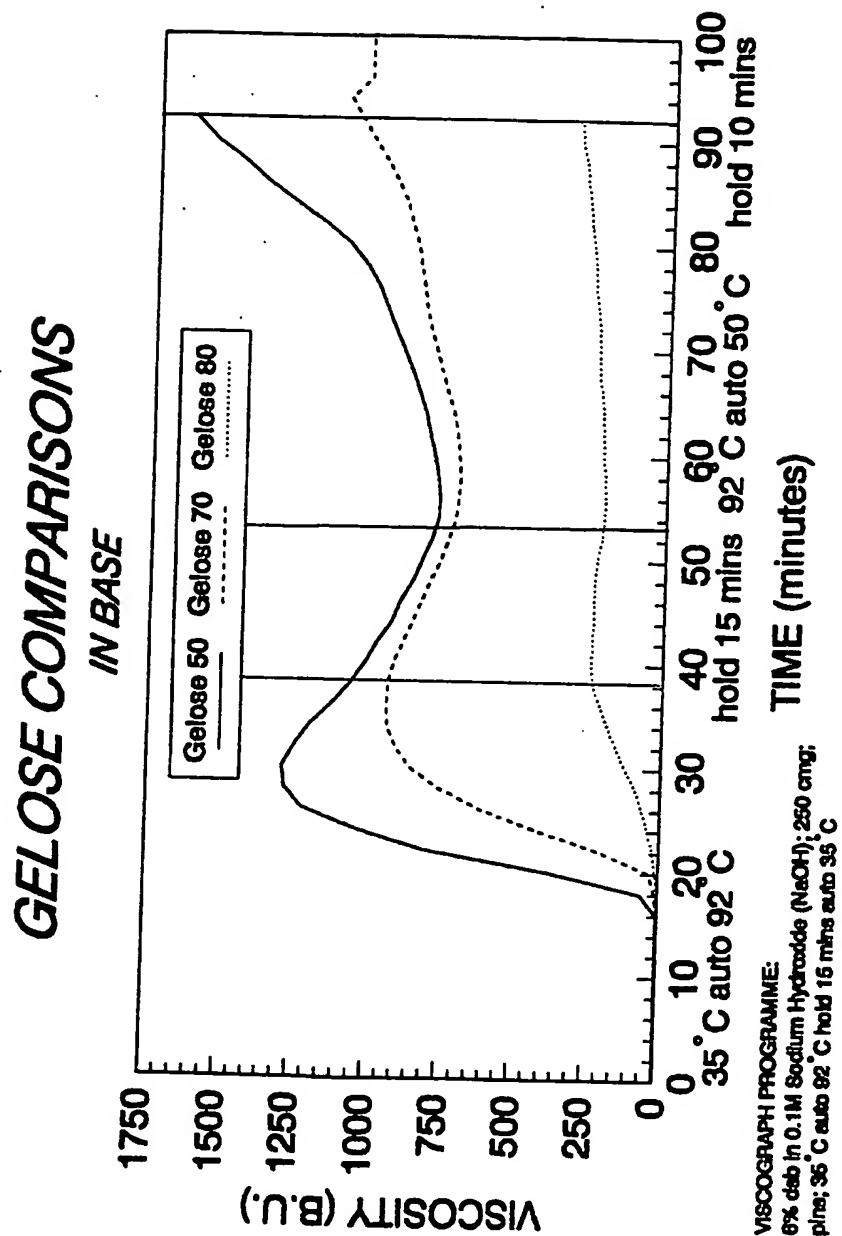


FIG. 3

4/4

**Total Dietary Fibre Content of High Amylose Maize Starch Fractions
High Amylose 80 (10/91)**

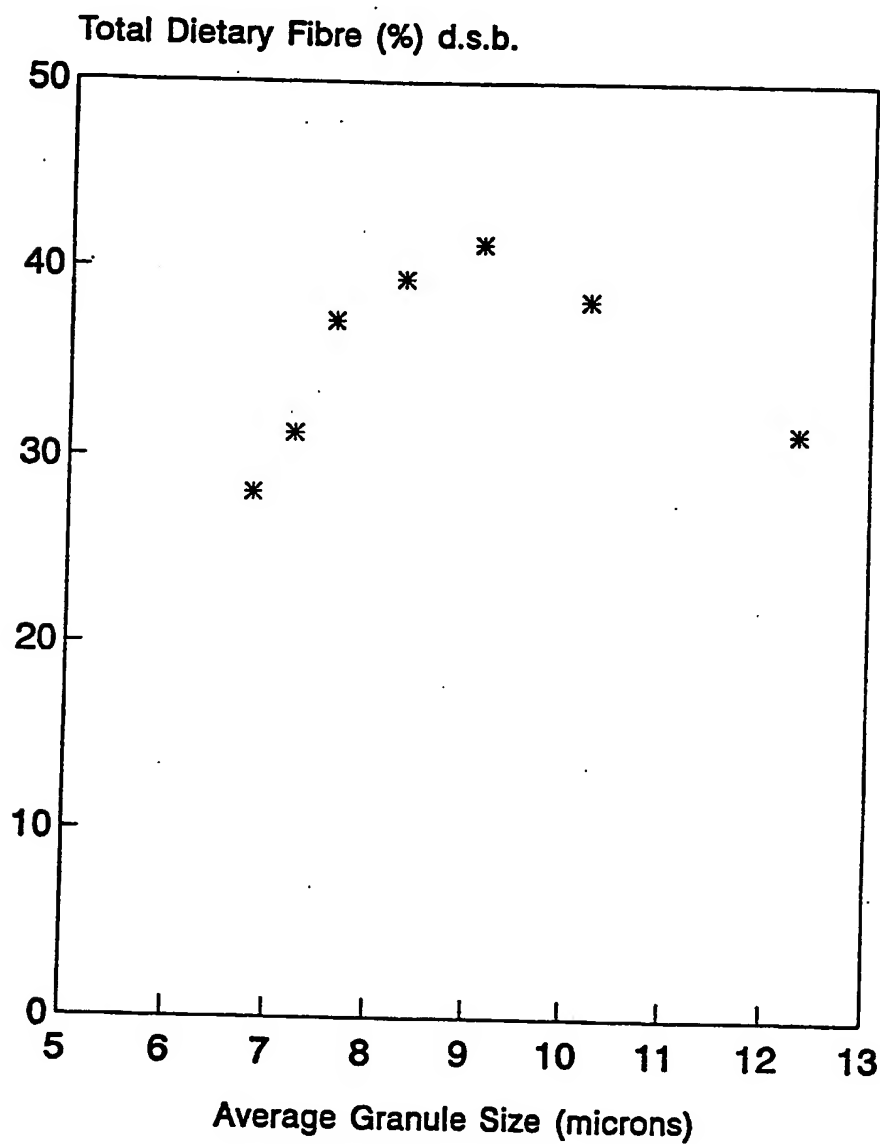


FIG. 4

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 93/00389

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl. ⁵ A01H 5/10, C08B 30/00, A23L 1/308, 1/0522				
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED				
Minimum documentation searched (classification system followed by classification symbols) IPC: A01H 5/10, C08B 30/00, A23L 1/308, 1/0522				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched IPC: AU as above				
Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) WPAT KEYWORDS - STARCH, HIGH (W) AMYLOSE, HYBRID, MAIZE, CORN, SEED, C08B CASM KEYWORDS - HYBRID, MAIZE, CORN, SEED, AMYLOSE, STARCH				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.		
X	Cereal Chemistry 68(6) 1991 pages 589-596 Szczodrak J. and Pomeranz Y. "Starch and Enzyme-Resistant Starch from High-Amylose Barley". Whole document.	16		
A	AU-B-45616/89 (625542) (IOWA STATE UNIVERSITY RESEARCH FOUNDATION INC) 31 May 1990 (31.05.90)	1-15		
<div style="display: flex; justify-content: space-between; align-items: center;"> <div style="display: flex; align-items: center;"> <input type="checkbox"/> Further documents are listed in the continuation of Box C. </div> <div style="display: flex; align-items: center;"> <input checked="" type="checkbox"/> See patent family annex. </div> </div>				
<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </td> </tr> </table>			<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>
<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>			
Date of the actual completion of the international search 1 October 1993 (01.10.93)		Date of mailing of the international search report 7 OCT 1993 (7.10.93)		
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929		Authorized officer <i>Mark Donaghey</i> MARK DONAGHEY Telephone No. (06) 2832414		

PCT/AU 93/00389

Patent Document Cited in Search Report		Patent Family Member					
AU	45616/89	EP US	372358 5004864	HU	896217	HU	63298
END OF ANNEX							